Synthesis of 7,8-(Methylenedioxy)-1-phenyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones as Novel and Potent Noncompetitive AMPA Receptor Antagonists

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A group of 7,8-(methylenedioxy)-1-phenyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones was synthesized and assayed for antagonism of rat brain α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors expressed in *Xenopus* oocytes. The benzodiazepinones inhibited AMPA-activated membrane current responses in a manner consistent with noncompetitive, allosteric inhibition of the receptor−channel complex. The most potent compound in the series was 1-(4-aminophenyl)-7,8-(methylenedioxy)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (**6**), which had an IC_{50} of 2.7 μM. For comparison, the reference compound GYKI 52466 (**2**) had an IC_{50} of 6.9 μM. Compound **6** also had potent anticonvulsant activity in a mouse maximum electroshock-induced seizure (MES) assay: the ED_{50} was 2.8 mg/kg iv, whereas the ED_{50} for GYKI 52466 was 4.6 mg/kg iv. In contrast to a previous report, the 7,8-dimethoxy analogue of **6** was a low-potency AMPA antagonist (IC_{50} >100 μM) and weak anticonvulsant (ED_{50} >10 mg/kg iv). The benzodiazepinones described herein are potent noncompetitive AMPA receptor antagonists that could have therapeutic potential as anticonvulsants and neuroprotectants.

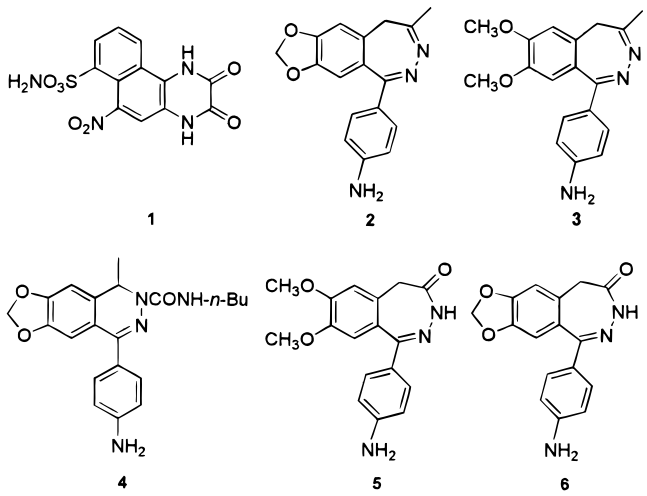
Introduction

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS), and glutamate receptors appear to play important roles in all aspects of CNS function.[1] Excessive stimulation of ionotropic glutamate receptors can, however, initiate a pathological cascade of events termed "excitotoxicity".[2] Excitotoxic processes are thought to cause much of the neuronal death produced in acute neurodegenerative disorders, and blockade of glutamate receptors is a commonly pursued strategy for drug discovery programs related to the treatment of stroke and traumatic brain injury (TBI).[3] Excitotoxicity and glutamate may also play a role in chronic neurodegenerative conditions such as Alzheimer's and Parkinson's disease.[4]

Ionotropic glutamate receptors are divided into three classes depending on their sensitivity to the selective agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid.[5] Among these, AMPA receptors are the principal mediators of fast excitatory neurotransmission and occur abundantly throughout the CNS.[6] The pivotal role of AMPA receptors in CNS function has prompted a search for AMPA receptor antagonists which could serve as therapeutically useful anticonvulsants and neuroprotectants.

The first types of AMPA receptor antagonist reported were competitive inhibitors such as NBQX (**1**) (Chart 1), PNQX, YM 90, and LY 293558.[7] Competitive AMPA antagonists have anticonvulsant properties in animals[8] and are efficacious as neuroprotectants in animal models of stroke, TBI, and spinal cord injury.[9] The latter studies even suggest that AMPA antagonists might have advantages over NMDA antagonists as neuroprotectants.[3,10] In particular, AMPA antagonists are active across a broader range of ischemia models than NMDA antagonists and do not induce psychotomimetic behaviors or direct neurotoxicity.

Chart 1



In 1993 a second type of AMPA receptor antagonist was reported.[11] These compounds are 2,3-benzodiazepines which inhibit AMPA receptor function by a noncompetitive, allosteric mechanism.[11] The prototypic compounds, e.g., GKYI 52466 (**2**), have anticonvulsant and neuroprotective properties in rodents.[8,12] Subsequently, 1,2-dihydrophthalazines, such as SYM 2207 (**4**),[13] and 2,3-benzodiazepin-4-ones, such as compound **5**,[14] were characterized as noncompetitive AMPA antagonists with potencies comparable to that of GKYI 52466.

Herein we report that substituted 7,8-(methylenedioxy)-1-phenyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-

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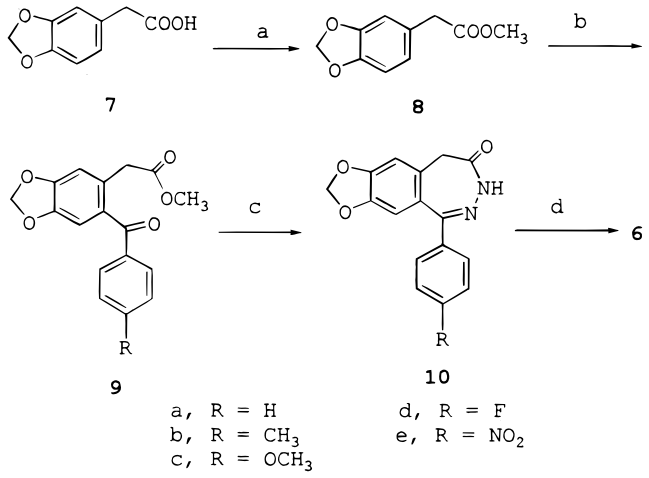
Scheme 1[a]



[a] (a) $MeOH/H_2SO_4$; (b) $SnCl_4/CH_2Cl_2$/4-R-PhCOCl or P_2O_5/$ClCH_2CH_2Cl$/4-R-$PhCO_2H$; (c) H_2NNH_2; (d) $CH_3CO_2H/H_2/Pd/C$.

ones, represented by compound **6**, are potent, systemically active, noncompetitive AMPA receptor antagonists.

Chemistry

As outlined in Scheme 1, reaction of 3,4-(methylenedioxy)phenylacetic acid (**7**) with methanol in the presence of sulfuric acid provided the corresponding methyl ester **8** in 86% yield. Friedel−Crafts acylation of **8** with various acyl chlorides 4-R-PhCOCl (R = **a**, H; **b**, CH_3; **c**, OCH_3; **d**, F) with $SnCl_4$ as the catalyst in CH_2Cl_2 furnished **9a**−**d** in 18−63% yields.[15] Compounds **9a**−**d** were refluxed with hydrazine hydrate in ethanol or propanol to afford the 2,3-benzodiazepin-4-ones **10a**−**d** in 24−52% yields.[16]

Reaction of ester **8** with 4-nitrobenzoyl chloride under the same conditions, however, did not give the desired compound **9e,** and the starting material **8** was recovered. Since it was reported that P_2O_5 can effect Friedel−Crafts reaction of acids with aromatic compounds,[17] ester **8** was refluxed with 4-nitrobenzoic acid in $ClCH_2CH_2Cl$ in the presence of P_2O_5 to provide **9e** in 50% yield. 2,3-Benzodiazepin-4-one **10e** was obtained by refluxing **9e** with hydrazine in ethanol. Reduction of the nitro group in **10e** via hydrogenation in acetic acid with Pd/C as the catalyst gave **6** in 68% yield.

Pharmacology

The inhibitory potency of 2,3-benzodiazepin-4-ones in vitro was assessed by measuring antagonism of AMPA-activated membrane current responses in *Xenopus laevis* oocytes expressing rat cerebral cortex poly(A)$^+$ RNA.[18] The mechanism of inhibition was investigated by looking at the effect of a fixed concentration of antagonist on the concentration−response curve for AMPA. Using standard techniques,[19] selected compounds were also tested for inhibition of AMPA-activated currents in cultured cortical neurons. The statistical significance of differences between IC_{50} values was tested by calculating pooled variances and the test statistic *t*.[20]

The anticonvulsant activity of the 2,3-benzodiazepin-4-ones was measured in vivo using a mouse maximum electroshock-induced seizure (MES) assay as a rough estimate of systemic bioavailability. Compounds were initially screened at 10 mg/kg iv (n = 8), with further evaluation of dose−response functions using 4−5 doses (n = 15−24/dose). The dose required to produce protection against tonic seizures in 50% of mice (ED_{50}) and the associated 95% confidence intervals were calculated based on the method of Litchfield and Wilcoxon.[21]

Table 1. In Vitro and In Vivo Potency of 2,3-Benzodiazepin-4-ones

entry	AMPA IC_{50},[a] μM	MES ED_{50},[b] mg/kg iv (95% CI)
10a	21.0 ± 1.9	12.9 (8.6−19.3)
10b	15.0 ± 0.70	16.0 (11.2−22.9)
10c	23.0 ± 2.5	NT[c]
10d	62.0 ± 4.8	>10
10e	56.0 ± 9.7	>10
6	2.7 ± 0.17	2.8 (2.2−3.6)
2	6.9 ± 1.2	4.6 (3.7−5.9)
5	180 ± 7	>10

[a] IC_{50} values were calculated from inhibition of AMPA-activated currents in *Xenopus* oocytes expressing rat cerebral cortex poly(A)$^+$ RNA. The 2,3-benzodiazepinones were assayed on responses elicited by 10 μM AMPA, i.e., currents constituting ∼80% of the maximum AMPA response. At a holding potential of −70 mV the average control current was ∼100 nA. Data are expressed as the mean ± SEM estimated by curve fitting to pooled data from 3 to 4 separate experiments (see Figure 1). [b] Seizures were induced in male NSA mice (20−25 g) by application of current (50 mA, 60 pulses/s, 0.8-ms pulse width, 1-s duration) using a Ugo Basile electroconvulsive treatment device (model 7801). Test compounds were administered in 10% Tween-80 vehicle, iv, 5 min before testing. Data are expressed as ED_{50} values with 95% confidence intervals calculated by Litchfield and Wilcoxon analysis.[21] [c] NT, not tested.

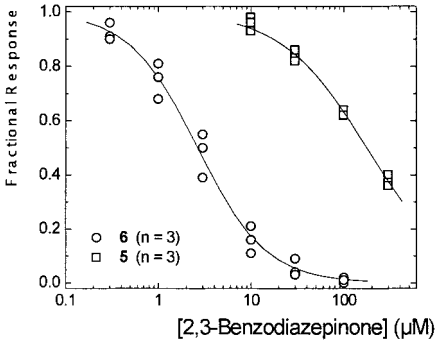


Figure 1. Inhibition of AMPA receptors by **5** and **6**. Membrane current responses were recorded in *Xenopus* oocytes expressing poly(A)$^+$ RNA isolated from rat cerebral cortex. Concentration−inhibition curves were measured on responses elicited by 10 μM AMPA. Curves are the best fits of pooled data to a four-parameter logistic equation.

Results and Discussion

Potencies for inhibition of AMPA-activated currents in oocytes of the 2,3-benzodiazepin-4-ones are given in Table 1. Sample concentration−inhibition data and curves are shown in Figure 1 for compounds **5** and **6**. The unsubstituted 2,3-benzodiazepin-4-one **10a** had moderate potency with an IC_{50} value of 21 μM. Substituting with electron-withdrawing groups, such as fluoro **10d** and nitro **10e**, at the 4-position of the benzene ring decreased potency to 62 and 56 μM, respectively. In contrast, substituting with the electron-donating group CH_3 (**10b)** slightly enhanced potency to 15 μM (p = 0.04). However, an OCH_3 substituent (**10c**), though a better electron-donating group than CH_3, did not increase potency. This argues that there is probably

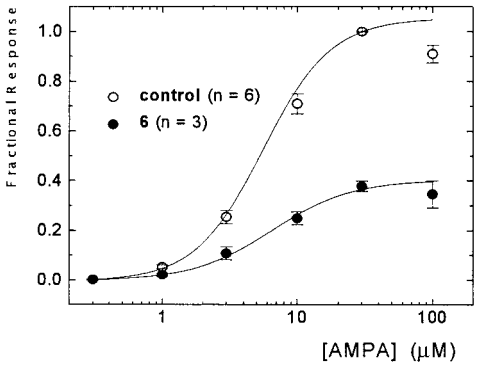


Figure 2. Noncompetitive inhibition of AMPA receptors by **6**. Membrane current responses were recorded in *Xenopus* oocytes expressing poly(A)$^+$ RNA isolated from rat cerebral cortex. Responses to AMPA were measured in the absence (control) and presence of **6** (5 μM). Concentration−response curves were fit to a four-parameter logistic equation.

a size-limited pocket at the 4-position. The amino group, a strong electron-donating substituent, resulted in the most potent 2,3-benzodiazepinone **6**, which had an IC_{50} of 2.7 μM. Tested side-by-side in oocytes, **6** was ~3-fold more potent than the reference compound GYKI 52466 (**2**), which had an IC_{50} of 6.9 μM ($p = 0.03$). Levels of inhibition produced by 5 μM **6** were essentially unaffected by changing the AMPA concentration from 3 to 100 μM (Figure 2). This is consistent with noncompetitive inhibition of AMPA receptor. The potency of **6** as an antagonist of neuronal AMPA receptors was checked by whole cell patch recordings from cultured rat cortical neurons. Assayed under steady-state conditions, **6** had an IC_{50} value on neuronal AMPA responses of 2.9 ± 0.2 μM ($n = 5$), almost identical to the value measured in the oocyte experiments. The IC_{50} of **2** on neuronal AMPA responses was 4.3 ± 0.4 ($n = 5$), again significantly weaker than **6** ($p = 0.01$). Using standard procedures, **6** (0.3−10 μM) was found to be inactive when assayed for modulation of recombinant human $GABA_A$ receptors ($\alpha_1\beta_2\gamma_{2L}$)[22] and rat NMDA (1A/2B)[19] receptors expressed in oocytes (data not shown).

Differing from a previous report,[14] the open-ring dimethoxy-2,3-benzodiazepin-4-one **5** had an IC_{50} of 180 μM on AMPA responses in oocytes (Figure 1), i.e., ~70-fold weaker than the corresponding methylenedioxy analogue **6** and ~25-fold less active than GYKI 52466 (**2**). Reasons for this discrepancy remain uncertain. In oocyte assays GYKI 52322 (**3**),[23] the dimethoxy analogue of GYKI 52466, is also a weak AMPA antagonist (IC_{50} > 200 μM). In the previous study,[14] the potency of **5** was estimated by electrical recordings from guinea pig brain slices, as opposed to oocytes. Yet, we see a close correlation between oocyte and neuronal data so this inconsistency does not conjure a ready explanation for differences in potency. Since the methylenedioxy ring is much smaller than the dimethoxy, the SAR of GYKI compounds and 2,3-benzodiazepin-4-ones suggested that there might be a size-limited pocket at the 7- and 8-positions of 2,3-benzodiazepines.

Compound **6** was found to exhibit anticonvulsant efficacy with an ED_{50} of 2.8 mg/kg iv (Table 1) in the MES assay. These effects were more potent than those of GYKI 52466 (**2**) which had an ED_{50} of 4.6 mg/kg. This appears to be a significant improvement in in vivo potency in that the 95% confidence intervals around the ED_{50}'s did not overlap. The unsubstituted and methyl-substituted benzodiazepine-4-ones, **10a**,**b**, respectively, were also active in the MES test, though their potencies were 4−6-fold lower than that of **6**. Compounds **5** and **10d**,**e** were inactive at 10 mg/kg iv and were not evaluated further. In general, anticonvulsant activity correlated well with potency at AMPA receptors. Again, there appears to be disagreement with a previous study where **5** was reported to have an ED_{50} of ~5 mg/kg ip in a mouse MES assay.[14] Ataxia was assessed for **2** and **6** using the hanging wire test.[24] The dose at which 50% of mice fell from the wire, defined as the toxic dose (TD_{50}), and the 95% confidence intervals were calculated. The therapeutic index (TI) was defined as the ED_{50}/TD_{50}. Compound **6** had a TD_{50} of 9.5 mg/kg (6.7−13.5), indicating a TI of 3.4. This compound thus displays a more favorable profile than **2**, which exhibited a similar TD_{50} of 10.3 mg/kg (8.5−12.5) resulting in a TI of 2.3.

Conclusion

In conclusion, 6,7-(methylenedioxy)-1-phenyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones are a structurally novel type of noncompetitive AMPA receptor antagonists. Compound **6** is a potent inhibitor which also has good activity in vivo as an anticonvulsant. These types of compounds may have therapeutic potential for the treatment of epilepsy and neurodegenerative disorders.

Experimental Section

Chemistry. All reagents were used as commercially received from Aldrich or Fluka. Methylene chloride was distilled from CaH_2, anhydrous $ClCH_2CH_2Cl$ ws used as received from Aldrich, and other solvents were used without further purification. The ^{1}H NMR spectra were recorded on a varian 300-MHz spectrometer. Chemical shifts are in ppm (δ), and coupling constants are in hertz. Melting points were measured on a MEL-TEMP II melting point apparatus and are uncorrected. Elemental analyses were performed by Dersert Analytics.

Methyl 3,4-(Methylenedioxy)phenylacetate (8). To a solution of 3,4-(methylenedioxy)phenylacetic acid (4.2 g, 23 mmol) in methanol (50 mL) was added concentrated sulfuric acid (1.5 mL). The mixture was refluxed for 48 h, cooled to room temperature, and neutralized with saturated $NaHCO_3$. The methanol was removed in vacuo. The residue was extracted with 1:1 hexane/EtOAc (2 × 100 mL). The combined organic phase was washed with water, saturated $NaHCO_3$, and brine and dried over Na_2SO_4. The solvent was removed in vacuo to yield the title compound as an oil (3.9 g, 86%): ^{1}H NMR ($CDCl_3$) 6.78−6.72 (m, 3H), 5.95 (s, 2H), 3.69 (s, 3H), 3.54 (s, 2H).

Methyl 2-Benzoyl-4,5-(methylenedioxy)phenylacetate (9a). To a solution of methyl 3,4-(methylenedioxy)phenylacetate (150 mg, 0.77 mmol) in CH_2Cl_2 (5 mL) was added $SnCl_4$ (1.0 M solution in CH_2Cl_2; 1.5 mL, 1.5 mmol) at 0 °C. Then benzoyl chloride (130 μL, 1.1 mmol) was added. The reaction mixture was allowed to warm to room temperature slowly. After 24 h, the mixture was added to a saturated $NaHCO_3$ solution (30 mL), diluted with 1:1 hexane/EtOAc (60 mL), washed with water and brine, and dried over Na_2SO_4. The solvent was removed in vacuo, and the resulting residue was purified by chromatography (4:1 hexane/EtOAc) to yield the title compound (110 mg, 48%): ^{1}H NMR ($CDCl_3$) 7.78−7.45 (m, 5H), 6.88 (s, 1H), 6.84 (s, 1H), 6.04 (s, 2H), 3.80 (s, 2H), 3.61 (s, 3H).

Compounds **9b**−**d** were prepared in a manner similar to that for **9a**.

Methyl 2-(4-Methylbenzoyl)-4,5-(methylenedioxy)phenylacetate (9b). From methyl 3,4-(methylenedioxy)pheny-

lacetate (319 mg, 1.64 mmol), CH_2Cl_2 (5 mL), $SnCl_4$ (1.0 M solution in CH_2Cl_2; 3.5 mL, 3.5 mmol), and 4-toluoyl chloride (260 μL, 1.97 mmol) was obtained **9b** as an oily solid (90 mg, 18%): ^{1}H NMR ($CDCl_3$) 7.69 (d, J = 8.0, 2H), 7.24 (d, J = 8.0, 2H), 6.88 (s, 1H), 6.83 (s, 1H), 6.03 (s, 2H), 3.78 (s, 2H), 3.60 (s, 3H), 2.43 (s, 3H).

Methyl 2-(4-Methoxybenzoyl)-4,5-(methylenedioxy)-phenylacetate (9c). From methyl 3,4-(methylenedioxy)-phenylacetate (244 mg, 1.26 mmol), CH_2Cl_2 (5 mL), $SnCl_4$ (1.0 M solution in CH_2Cl_2; 2.5 mL, 2.5 mmol), and 4-anisoyl chloride (220 μL, 1.62 mmol) was obtained **9c** as a white solid (110 mg, 27%): ^{1}H NMR ($CDCl_3$) 7.78 (d, 2H, J = 8.6), 6.93 (d, 2H, J = 8.6), 6.86 (s, 1H), 6.83 (s, 1H), 6.03 (s, 2H), 3.88 (s, 3H), 3.75 (s, 2H), 3.59 (s, 3H).

Methyl 2-(4-Fluorobenzoyl)-4,5-(methylenedioxy)phenylacetate (9d). From methyl 3,4-(methylenedioxy)phenylacetate (340 mg, 1.75 mmol), CH_2Cl_2 (8 mL), $SnCl_4$ (1.0 M solution in CH_2Cl_2; 3.5 mL, 3.5 mmol), and 4-fluorobenzoyl chloride (270 μL, 2.28 mmol) was obtained **9d** as a solid (350 mg, 63%): ^{1}H NMR ($CDCl_3$) 7.81 (m, 2H), 7.13 (m, 2H), 6.85 (s, 1H), 6.84 (s, 1H), 6.04 (s, 2H), 3.79 (s, 2H), 3.61 (s, 3H).

Methyl 4,5-(Methylenedioxy)-2-(4-nitrobenzoyl)phenylacetate (9e). To a solution of methyl 3,4-(methylenedioxy)-phenylacetate (5.4 g, 28 mmol) in $ClCH_2CH_2Cl$ (100 mL) were added 4-nitrobenzoic acid (7.2 g, 43 mmol) and P_2O_5 (18 g) at room temperature under argon. The mixture was refluxed for 28 h, cooled to room temperature, and then diluted slowly by addition of cold water. The resulting mixture was carefully neutralized with solid K_2CO_3 and extracted with 1:1 hexane/EtOAc (2 × 300 mL). The combined extracts were washed with water and brine and dried over Na_2SO_4. The solvent was removed in vacuo, and the resulting residue was purified by chromatography (3:1 hexane/EtOAc) to afford the title compound as a yellow solid (4.7 g, 14 mmol, 50%): ^{1}H NMR ($CDCl_3$) 8.32 (d, J = 8.5, 2H), 7.92 (d, J = 8.5, 2H), 6.85 (s, 1H), 6.81 (s, 1H), 6.07 (s, 2H), 3.88 (s, 2H), 3.63 (s, 3H).

7,8-(Methylenedioxy)-1-phenyl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (10a). A solution of methyl 2-benzoyl-4,5-(methylenedioxy)phenylacetate (110 mg, 0.37 mmol) and hydrazine hydrate (30 μL, 0.53 mmol) in ethanol (15 mL) was refluxed for 5 days. The solvent was removed in vacuo, and the resulting residue was purified by chromatography to yield the title compound (30 mg, 0.11 mmol, 29%): mp 182−184 °C; ^{1}H NMR ($CDCl_3$) 8.59 (s, 1H), 7.60−7.41 (m, 5H), 6.83 (s, 1H), 6.63 (s, 1H), 6.03 (s, 2H), 3.46 (s, 2H). Anal. ($C_{16}H_{12}N_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

Compounds **10b**−**d** were prepared in a manner similar to that of **10a**.

7,8-(Methylenedioxy)-1-(4-methylphenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (10b). From methyl 4,5-(methylenedioxy)-2-(4-methylbenzoyl)phenylacetate (90 mg, 0.29 mmol), hydrazine hydrate (50 μL, 0.88 mmol), and acetic acid (40 μL) in ethanol (10 mL) was obtained **10b** as a solid in 52% yield: mp 222−224 °C; ^{1}H NMR ($CDCl_3$) 8.36 (s, 1H), 7.48 (d, J = 8.0, 2H), 7.23 (d, J = 8.0, 2H), 6.82 (s, 1H), 6.64 (s, 1H), 6.02 (s, 2H), 3.45 (s, 2H), 2.41 (s, 3H). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

1-(4-Methoxyphenyl)-7,8-(methylenedioxy)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (10c). From methyl 4,5-(methylenedioxy)-2-(4-methoxybenzoyl)phenylacetate (102 mg, 0.31 mmol) and hydrazine hydrate (30 μL, 0.53 mmol) in 1-propanol (8 mL) was obtained **10c** as a solid in 24% yield at 31% conversion: mp 194−196 °C; ^{1}H NMR ($CDCl_3$) 8.48 (s, 1H), 7.55 (d, J = 8.8, 2H), 6.94 (d, J = 8.8, 2H), 6.83 (s, 1H), 6.66 (s, 1H), 6.03 (s, 2H), 3.86 (s, 3H), 3.44 (s, 2H). Anal. ($C_{17}H_{14}N_2O_4$) C, H, N.

1-(4-Fluorophenyl)-7,8-(methylenedioxy)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (10d). From methyl 2-(4-fluorobenzoyl)-4,5-(methylenedioxy)phenylacetate (350 mg, 1.11 mmol), hydrazine hydrate (180 μL, 3.18 mmol), and acetic acid (50 μL) in ethanol (10 mL) was obtained **10d** as a solid in 31% yield: mp 205−207 °C; ^{1}H NMR ($CDCl_3$) 8.45 (s, 1H), 7.59 (dd, J = 8.5, 5.5, 2H), 7.11 (dd, J = 8.5, 8.5, 2H), 6.83 (s, 1H), 6.61 (s, 1H), 6.04 (s, 2H), 3.45 (s, 2H). Anal. ($C_{16}H_{11}FN_2O_3 \cdot {}^1/_8H_2O$) C, H, N.

7,8-(Methylenedioxy)-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (10e). A mixture of methyl 4,5-(methylenedioxy)-2-(4-nitrobenzoyl)phenylacetate (20 g, 58 mmol) and hydrazine hydrate (7 mL, 124 mmol) in ethanol (150 mL) was refluxed for 5 h. Then 6 N HCl (10 mL) was added, and the mixture was further refluxed for 3 h. After cooling, the resulting yellow solid was collected by filtration, washed with water, and dried in vacuo to yield the title compound (10.5 g, 32 mmol, 55%) as a solid: mp 293−295 °C; ^{1}H NMR (DMSO-d_6) 11.2 (s, 1H), 8.31(d, J = 8.8, 2H), 7.79 (d, J = 8.8, 2H), 7.13 (s, 1H), 6.65 (s, 1H), 6.12 (s, 2H), 3.45 (s, 2H). Anal. ($C_{16}H_{11}N_3O_5$) C, H, N.

1-(4-Aminophenyl)-7,8-(methylenedioxy)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (6). To a suspension of 7,8-(methylenedioxy)-1-(4-nitrophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepin-4-one (1.3 g, 4.0 mmol) in acetic acid (30 mL) was added 5% Pd/C (130 mg). The mixture was shaken under hydrogen (40 psi) for 18 h. The Pd/C was filtered out. The filtrate was concentrated, diluted with 2 N HCl (50 mL), washed with EtOAc (3 × 30 mL), neutralized with 2 N NaOH, and extracted with EtOAc (150 mL). The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to yield the title compound as a yellow solid (0.8 g, 2.7 mmol, 68%): mp 242−244 °C; ^{1}H NMR ($CDCl_3$) 8.32 (s, 1H), 7.41 (d, J = 8.5, 2H), 6.81 (s, 1H), 6.70 (s, 1H), 6.68 (d, J = 8.5, 2H), 6.02 (s, 2H), 3.94 (brs, 2H), 3.42 (s, 2H). Anal. ($C_{16}H_{13}N_3O_3 \cdot {}^1/_4H_2O$) C, H; N: calcd, 14.02; found, 13.48.

Electrophysiology. Total RNA was prepared from rat cerebral cortex by homogenization in 6 M urea/3 M LiCl followed by phenol/chloroform extraction. Polyadenylated (poly(A)$^+$) RNA was isolated from total cellular RNA by oligo-dT cellulose chromatography. *Xenopus* oocytes were prepared for injection by the method of Woodward et al.[18] Oocytes were microinjected with approximately 25 ng of cortical poly(A)$^+$ RNA and stored in Barth's medium (containing in mM: NaCl, 88; KCl, 1; $CaCl_2$, 0.41; $Ca(NO_3)_2$, 0.33; $MgSO_4$, 0.82; $NaHCO_3$, 2.4; HEPES, 5; pH 7.4, with 0.1 mg/mL gentamycin sulfate) for 4−16 days prior to recording. Membrane current responses were recorded in frog Ringer solution containing (in mM): NaCl, 115; KCl, 2; $CaCl_2$, 1.8; HEPES, 5; pH 7.4. Electrical recordings were made using a conventional two-electrode voltage clamp (Dagan TEV-200). The oocyte was placed in a 5-mL chamber lined with nylon mesh, impaled with two microelectrodes and voltage-clamped at a holding potential of −70 mV. A microcapillary linear array system was used to superfuse the oocyte in Ringer solution and to apply drugs and wash solutions. A control concentration (10 μM) of AMPA was applied to the oocyte to determine a baseline membrane current response. The inhibition of this response by 2,3-benzodiazepin-4-ones was measured by applying increasing concentrations of the antagonist in Ringer solution for ∼30 s followed by coapplication of the inhibitor with 10 μM AMPA. The resulting membrane current responses were fit to a four-parameter logistic equation (Origin, Microcal Software, Inc.). All electrophysiological data are expressed as mean ± standard error of the mean (SEM) to two significant figures.

In Vivo Pharmacology. Male NSA mice weighing between 15 and 20 g were obtained from Harlan Sprague−Dawley (San Diego, CA). Compounds were dissolved/suspended in Tween 80 (10%)/distilled water (90%), and were placed in solution/suspension by warming and sonication for 1−4 h. Solutions were prepared on a weight/volume basis on the day of or evening prior to use. Compounds were administered intravenously (iv). All drugs were administered in volumes of 200 mL/20 g. Seizures were induced by application of current (50 mA, 60 pulses/s, 0.8-ms pulse width, 1-s duration, d.c.) using a Ugo Basile electroconvulsive treatment device (model 7801). Mice were restrained by gripping the loose skin on their dorsal surface, and saline-coated corneal electrodes were held lightly against the two corneas. Current was applied and animals were observed for a period of up to

30 s for the occurrence of a tonic hindlimb extension in excess of 90° from the plane of the body. The hanging wire test has been described previously[24] and used a custom built apparatus that consisted of a metal wire (2-mm diameter) suspended horizontally above the benchtop (25 cm). Mice were held by the base of the tail, their forepaws placed in contact with the wire, and then released. Animals were required to bring at least one hindpaw in contact with the wire within 10 s in order to be scored as a pass. The dose of drug required to produce an anticonvulsant effect (ED_{50}) or motor impairment (TD_{50}) in 50% of animals and its associated 95% confidence limits were calculated by the method of Litchfield and Wilcoxon[21] using a commercial computer program (PHARM/PCS v4.2, MicroComputer Specialists). The protective index (PI) was calculated by dividing the TD_{50} by the ED_{50}.

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